

EFFECTS OF EARLY CIRRHOSIS ON HEPATIC VASCULAR
RESISTANCE IN THE RAT

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An abstract of a Thesis by
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The problem. Increased vascular resistance in the liver is a common symptom of established cirrhosis of the liver. This investigation measured changes in resistance during induction of cirrhosis, to determine whether the increase in vascular resistance might be part of the mechanism by which cirrhosis develops.

Procedure. Early cirrhosis was induced in rats by injections of C Cl₄ and olive oil over a 35 day period. The livers were excised and perfused with aerated Locke's solution through the hepatic portal vein, using 13 different flow rates. Portal pressures at these flow rates were recorded continuously. Resistances were calculated as the ratio of pressure to flow rate, for each of three groups of livers: those from uninjected control rats, those from rats injected with olive oil, and those from rats injected with olive oil and C Cl₄.

Findings. Resistance was found to be significantly higher in livers of rats injected with olive oil or with olive oil plus C Cl₄, than in livers of uninjected control rats. The difference was especially noticeable at the lower flow rates.

Conclusions. It is clear that increased vascular resistance is not merely a late side-effect of cirrhosis.

Recommendations. More detailed studies should be made to describe the changes in resistance during induction of cirrhosis, and to establish the structural basis of this increased resistance.

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INTRODUCTION AND REVIEW OF THE LITERATURE

Much work has been done in the separate areas of liver regeneration, cirrhosis, and portal hypertension. An assimilation of information and ideas from all three areas is needed to arrive at a better understanding of how the liver repairs itself, and perhaps of how a cirrhotic liver could be helped to return to normal. A step in that direction would be to see whether there is increased vascular resistance in early cirrhotic rats.

Cirrhosis of the liver can be defined by the following criteria: proliferation of connective tissue, degeneration and death of cells, nodular regeneration of parenchymal tissue, tawny color of the liver, and fibrosis with scarring. Also present are regeneration nodules and portal hypertension, although in later stages even regeneration will not take place. Cirrhosis can be experimentally induced in animals by injections of poisons such as C Cl_4 , by breathing vapors of C Cl_4 , or by diet deficiencies. In humans it often is caused by drinking too much alcohol for too long.

There is much descriptive literature available on the normal architecture and function of the liver (Elias and Sherrick, 1969). The changes during regeneration after surgery or acute injury are summarized by Bucher and Malt (1971). Islami, Pack, and Hubbard (1958) found that a normal rat liver regenerated fully when a partial hepatectomy was done.

In an early cirrhotic liver when no more injections were given to further the cirrhosis, the liver regenerated nicely. If a partial hepatectomy was performed, return to normal was more rapid. A fully cirrhotic rat liver with no more injections for further poisoning did not return to normal, but after a partial hepatectomy the liver returned to normal as judged by gross and microscopic appearance. In other words after a certain point in cirrhosis no regeneration takes place, and the condition of the liver worsens unless a partial hepatectomy is done. Rabinovici and Wiener (1961) showed the same results, also in rats. However, Lin and Chen (1965) found that while normal human livers regenerate after partial removal cirrhotic ones do not, even with partial hepatectomy. Therefore in human cirrhosis, regeneration by way of a partial hepatectomy is not the answer.

The portal hypertension so often found with cirrhosis may possibly be due to the incomplete regeneration that occurs. Kelty, Baggenstoss, and Butt (1950) observed regeneration nodules which cause a distortion of the vascular system and could lead to portal hypertension. Baggenstoss (1955) reemphasizes this. On the other hand, MacDonald (1962) feels the regeneration nodule is not a main step in cirrhosis or hypertension, but that vascular changes have a more important role. Rogers and MacDonald (1965) also argue this. Popper, Elias, and Petty (1952) observed anastomoses or shunts between the portal vein and hepatic vein with blood from the

portal veins bypassing the nodular and lobular sinusoids. These nodules were supplied only by the hepatic artery. The portal hypertension could be caused by partial obliteration of the vascular bed or by the transmission of arterial pressure into the vascular bed. This kind of portal hypertension was also observed by Kruezer, Schueller, and Schenk (1972) in dogs. They found a decrease in blood flow to the liver, and also that one-fourth of the blood to the liver was supplied by the hepatic artery. But in cirrhosis the arterial portion increased to one-third, with an increase in portal pressure. This increased hepatic artery flow might be due to development of presinusoidal anastomoses between the hepatic arterioles and the portal venous radicals, perhaps an adaptive response to supply oxygen to the regenerating lobules. Normally three-fourths of blood is supplied by the portal vein. Peters and Womack (1961) proposed that the hypertension of cirrhosis comes from an increase in portal venous flow due to augmented arteriovenous connections in the regions drained by the portal vein. Reynolds, Hildemura, Michel, and Peters (1969) also noted an increase in collagen in the central portion of the liver lobule, which could cause hypertension by blocking sinusoids or reducing their diameter even more so than the regeneration nodule. Leevy (1965) reviews most of these ideas presented above.

Not only is it uncertain just what is the physical explanation for portal hypertension; it also is unclear just

how it is related to the persistence of cirrhosis. Banerjee and Aikat (1968) found the portal pressure of normal hepatectomized rats went up after the hepatectomy and over a few months came back down close to normal. The collagen content did the same thing. In rats with cirrhosis the portal pressure was high and, with no more injections to continue inducing cirrhosis, dropped about one-half of maximum, but was still high. The collagen content followed the same pattern. In cirrhotic hepatectomized rats, the portal pressure was high and stayed that way with a slight decrease, whereas the collagen content was high and dropped closer to normal with time. It is possible that portal hypertension is not merely a late side effect of cirrhosis, but rather is part of the cause of cirrhosis. If normal regeneration is controlled by a circulating stimulating factor in the portal blood (Bucher and Malt, 1971), decreased portal blood flow due to hypertension would result in failure of the liver to be sufficiently stimulated to regenerate.

No one is sure exactly in what order injury to the liver takes place in cirrhosis induction, how or why a liver regenerates the way it does, or how portal hypertension is related to cirrhosis. Somehow all three areas must be taken as a whole, information assimilated, and then work done to come up with a cure for cirrhosis. A good place to begin would be to perfuse a cirrhotic liver, measure the vascular resistance, find the structural reason for any change, and

then find how that can be corrected. This study was designed to test whether hepatic vascular resistance is increased early in the induction of cirrhosis, and so might be part of the causative process.

MATERIALS AND METHODS

Treatments to induce cirrhosis of the liver were given to 6 month old male rats (Sasco, Omaha, Nebraska). They were kept three to a cage, and watered and fed Purina Laboratory Chow daily. The following groups were used: 17 injected with C Cl₄ in olive oil, 9 injected with olive oil, and 6 uninjected controls. Intramuscular injections of 0.1 ml C Cl₄ and 0.9 ml olive oil were given every other day for 30 days to produce early cirrhosis. After 31 days the dosage was increased to 0.12 ml C Cl₄ and 0.88 ml olive oil every other day until days 35 and 36. Olive oil controls received injections of 1 ml of commercial olive oil every other day. This method of producing cirrhosis was that of Islami, Pack, and Hubbard (1958).

On day 35 or 36 of the injections the rats were killed and the livers removed. Each liver was perfused by the following method. Aerated Locke's solution (without glucose) was put in beakers in a water bath at 37° C. The solution was pumped by a Model T-8 finger pump (Sigmamotor Company, Middleport, New York) through Tygon tubing (I.D. 1/8"). Unit pump settings of 2-14 were used to perfuse the

liver at different flow rates. Pressure was measured with a Statham blood pressure transducer #P23AA (Statham Laboratories Inc., Hato Rey, Puerto Rico), calibrated at static pressures by a water manometer and recorded on a Beckman Type R-411 dynograph. Fluid was pumped through a polyethylene cannula inserted in the hepatic portal vein of an excised liver. The Locke's solution, water manometer, transducer, and liver were all set at the same height to avoid static pressure differences.

Rats were killed with a blow on the head and decapitated. The vena cava was severed. While a fluid flow of 6 ml/min was pumped through it, the cannula was inserted into the hepatic portal vein and tied in place. The liver was then excised, and placed in a shallow bowl; the heights were adjusted as mentioned previously. After a few minutes of pumping at this rate to clear the liver of any unwanted blood, pressures were recorded, starting at a flow rate of 1 ml/min and increasing to 55.5 ml/min. In a few cases, pressures were redetermined at the lower flow rates after the series of high flow rates had been conducted. This was done to see if any major damage had been done to the liver as the flow rate had increased. Pressure readings were then read and recorded for future use.

The flow rates at different pump settings had been calibrated at a range of back pressures exceeding the range used for perfusion. The flow rates did not vary with the

pressures within the accuracy of the device for measurement. Resistance was therefore then calculated by dividing the pressure by the flow rate. Means for 3 olive oil controls, 6 controls, and 9 injected with C Cl₄ were then calculated and graphed with the flow rate on the x-axis and the resistance on the y-axis. This data was then used for regression analysis by comparing the slopes of the 3 lines at 95% confidence level.

DATA AND DISCUSSION

Figure 1 shows graphically the relationship between the resistance and flow rate for the 13 unit pump settings of flow rates. The resistances represent means of data from 6 uninjected control rats, 3 rats injected with olive oil, and 9 rats injected with C Cl₄ and olive oil. The curves for resistance are very similar for the rats injected with olive oil and those injected with C Cl₄ and olive oil. Both have very high resistances at flow rates 1 to 15 ml/min. Data from both are noticeably different from that of the uninjected controls which starts with a very low resistance and gradually increases for flows up to 31.5 ml/min. From 15 to 31.5 ml/min there is a more gradual decrease in the resistances of the rats injected with olive oil and those injected with C Cl₄ and olive oil. From 31.5 to 55.5 ml/min both sets of resistances change very little. In the uninjected controls the resistance does not change from 31.5 to 55.5 ml/min but still

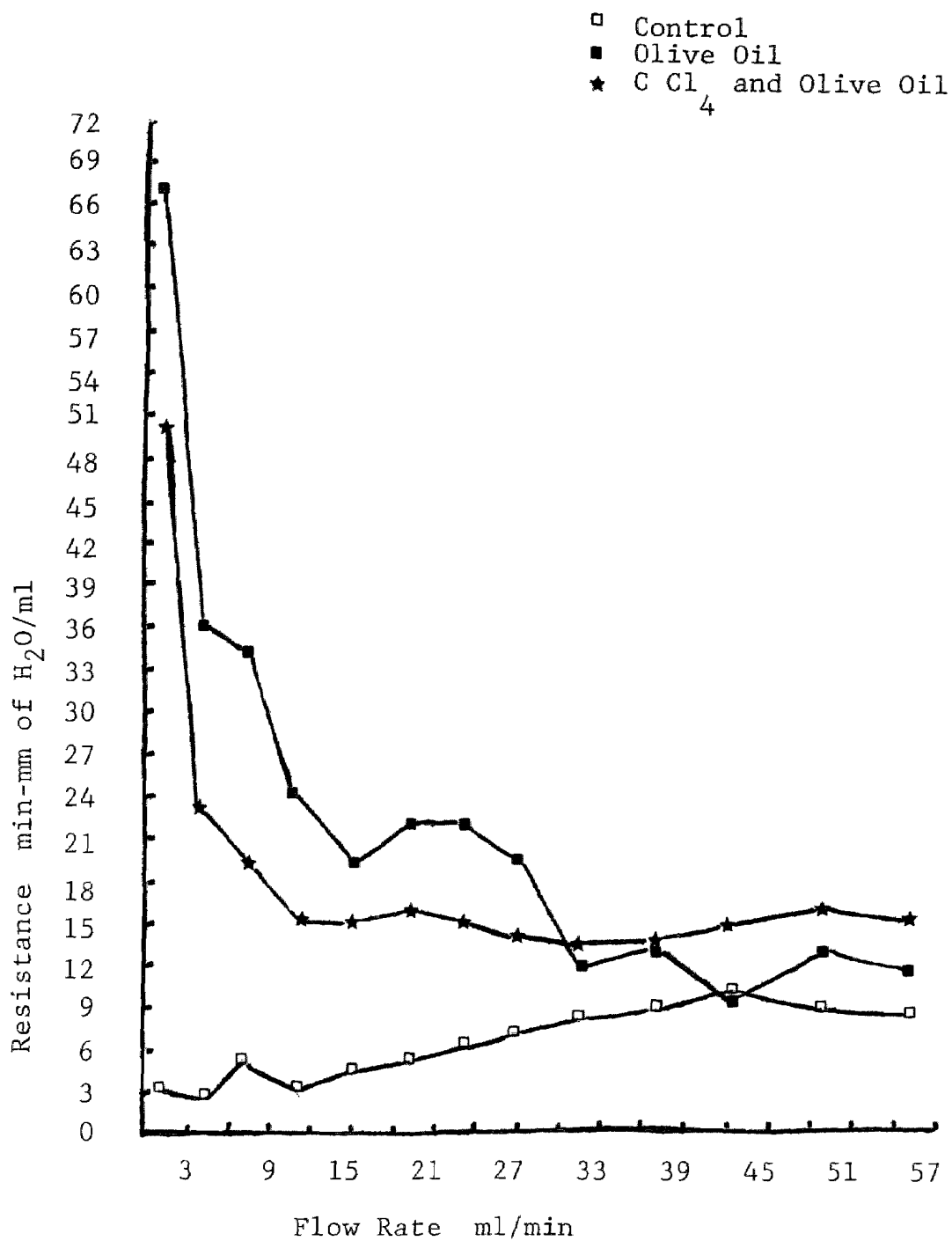


Figure 1. Average hepatic vascular resistance of 6 uninjected control rats, 3 rats injected with olive oil and 9 rats injected with C Cl₄ and olive oil plotted with corresponding flow rates.

is noticeably different from both types of injected rats. After completion of the first flow rate four complete reruns were taken along with four partial reruns starting at 1 ml/min and working up to 55.5 ml/min just as in the original readings. Resistance during reruns of control rat livers were about 5 units higher in the early flow rates but about 4 units at the higher flow rates. Resistance during reruns of livers from rats receiving olive oil were about the same as the original readings and that from rats receiving C Cl₄ and olive oil were lower to about the same.

Regression analysis was done on all three lines in Figure 1 and the results are shown in Table 1. Using the t-test, confidence intervals at the 95% level were then established for the m, or slope value of each line. It can be seen from Table 1 that the rats injected with olive oil and those with C Cl₄ and olive oil have an overlap in confidence intervals of slope values. This means the two lines are very similar. On the other hand, the uninjected control slope plus or minus standard error value does not overlap either of the other two, thus meaning it is different from these 2 lines. At the right is the complete regression-line equation. As these equations show, the lines are quite different: the controls have a positive slope and a low y-intercept, whereas the other two lines have negative slopes and high y-intercepts.

Table 1. Confidence intervals at the 95% level of the slope of the 3 regression-lines for hepatic vascular resistance in 6 uninjected control rats, 3 rats injected with olive oil and 9 rats injected with $C Cl_4$ and olive oil.

	m \pm std. error	regression- y=mx+b line equation
Control	0.12 \pm 0.08	y=0.12x+2.86
Olive oil	-0.65 \pm 0.36	y=-0.65x+39.92
$C Cl_4$ and olive oil	-0.31 \pm 0.14	y=-0.31x+25.95

This method for studying cirrhosis is new and produces good consistent data. The data is easily obtained, read, and double checked. The results can be reproduced very easily. One rat can produce a large number of results because pressures are checked at a wide range of flow rates and not just at the average flow rate of the rat's liver which is 23 to 31.5 ml/min. The data from reruns was quite similar, which means the method is not destructive to the liver. With the number of rats and the range of flow rates involved it is very easy to show the differences or similarities in the resistance of the three groups. Regression analysis can be done and results easily checked and compared.

Obvious conclusions which can be drawn from the data are that olive oil, and olive oil and $C Cl_4$ have indistinguishable effects on the resistance of the liver. At a flow rate of 1 to 15 ml/min livers from both treatments have a very

high resistance; the sinusoids do not readily open up at lower flow rates. The blood could be flowing through the healthy sinusoids which open easily as shown by the low resistance in the uninjected controls, or the blood could be being shunted to the healthy sinusoids with not much going through the ones with regeneration taking place in them or with collagen fibers in them. From 15 to 31.5 ml/min both have a decrease in resistance which means the sinusoids are opening up even though the resistance is still higher than in the controls. From 31.5 to 55.5 ml/min the resistances level off with as many sinusoids open as possible, but there still is a higher resistance than in controls. In the controls, which have a much lower and different resistance pattern, the sinusoids open readily at lower flow rates as shown by the low resistance. The resistance slowly increases until at 31.5 to 55.5 ml/min it levels off still significantly below that of the experimentals. All sinusoids are open and there is a lower resistance because of no obstruction in the sinusoids as compared with the experimentals. The above ideas on sinusoids opening up and having a low resistance come from the fact that resistance in a liver and its sinusoids is similar to a parallel electrical circuit; the more sinusoids that are open the less the overall resistance, which also will cause a decreased pressure instead of an increased one. In laminar flow through a tube the resistance at a constant flow rate is inversely proportional to the fourth power of the radius of the tube. The

reason that resistance in this experiment was higher at a lower flow rate must have been that the sinusoids had not opened wider, whereas at the higher flow rates the radius was larger in the sinusoids, and the pressure and resistance is less proportionately. Possibly because of injury to the sinusoids, formation of regeneration nodules, or collagen deposition, the radius is constricted in the experimentals thus causing an increased resistance as well as an increased pressure. I believe early cirrhosis was produced in the rats injected with $C Cl_4$ and olive oil (Islami, Pack, and Hubbard, 1958) as was evident by the tawny color and bumpy appearance of the livers. The livers of rats injected with olive oil appeared normal externally, but their resistance and pressures were similar to those of the rats injected with $C Cl_4$ and olive oil. This could be caused by fat globules accumulating in the liver cells and swelling into the sinusoids due to the olive oil injected into both sets of rats.

Possible causes of portal hypertension are discussed in many places in the literature. One which should be disregarded as a sole cause is that of Peters and Womack (1961), who feel the portal hypertension comes not from an increased resistance but from an increased portal venous flow as well as more arteriovenous connections. This could cause some increased portal pressure but the results in this study have shown a definite increase in resistance. Also,

how can arterial pressure getting into the sinusoids be the explanation for portal hypertension when no arterial flow was used and still had hypertension? It can not. Another possible cause of the portal hypertension (Popper, Elias, and Petty, 1952) could be the bypassing of the nodular and lobular sinusoids by the blood because of anastomoses, with partial obliteration of the vascular bed by transmission of arterial pressure into the vascular bed. These shunts could be formed because of the distortions taking place in the vascular bed (MacDonald, 1962). The distortions would cause an increased resistance, causing the shunts to be formed. The above idea could be one explanation for the hypertension and high resistance in this study for both the rats injected with olive oil and those injected with $C Cl_4$ and olive oil. Regeneration nodules (Kelty, Baggenstoss, and Butt, 1950) can also distort the vascular bed. They are formed in an early cirrhotic liver as a result of the death of cells poisoned by $C Cl_4$. New cells form to replace the dead ones; the randomness of the proliferation of these cells can constrict and change blood flow in the sinusoids a great deal. The regeneration nodules are probably not an explanation for the increased resistance in this study as an equally high resistance with both the rats injected with olive oil and those injected with $C Cl_4$ and olive oil was found. There should not be any necrosis or regeneration nodules in the rats injected with olive oil. With an increase in the

number of cells there is an increase in connective fibers made of collagen as shown by Rabinovici and Wiener (1961) and Banerjee and Aikat (1968). These fibers could collect in and around the sinusoids and restrict passage of blood through them causing an increased resistance and thus an increased portal pressure. Reynolds, Hidemura, Michel, and Peters (1969) also believe portal hypertension is due to an increased vascular resistance as they found collagen fibers deposited in the sinusoids of livers with cirrhosis and without cirrhosis but both groups had portal hypertension. The collagen could account for the increased vascular resistance and thus hypertension in the rats injected with C Cl_4 and olive oil. The hypertension in the rats injected with olive oil could come from the fat globules accumulating in the liver cells and swelling into the sinusoids, or just in the sinusoids, and therefore blocking the sinusoids and increasing vascular resistance.

Another factor to be considered in hypertension is the portal blood factor (Bucher and Malt, 1971). This is a substance carried in the portal stream which stimulates regeneration in the liver. If blood is shunted away from the regenerating cells there would be no stimulus to cause further regeneration and return to normal. Also with cirrhosis there is usually a decrease of blood flow to the liver, especially by the hepatic portal vein, which would give the liver less portal blood factor. Both these ideas

would reduce the regeneration and cause more resistance and therefore more hypertension. Hypertension could then be a cause of cirrhosis or a result of cirrhosis or both.

CONCLUSIONS

Hepatic vascular resistance can be satisfactorily measured by the method here introduced. Rats injected with C Cl_4 and olive oil have a much higher hepatic vascular resistance than do uninjected control rats. This is especially true at low flow rates. The rats injected with olive oil also have a higher resistance than uninjected control rats. It is very similar to the rats injected with C Cl_4 and olive oil.

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